

## **A reappraisal of nitrogen-to-protein conversion factors in cassava roots**

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A study was conducted comparing the amino acid profiles of 10 commercial varieties (COL) and 15 clones with the highest levels of nitrogen (HIN) over two years of harvests. The highest nitrogen values detected in roots to date are close to 1.30%. The conversion factors of total nitrogen into protein calculated from amino acid profiles was  $3.6 \pm 0.9$  for COL and  $2.8 \pm 0.2$  for HIN. 53.7% of the total nitrogen is measured from protein with no difference between COL and HIN. The remaining 46.3% corresponds to ammonium ions, nucleic acids, or other nitrogenous non-protein molecules. Nitrogen content is  $15.1 \pm 1.2$  and  $19.1 \pm 0.6\%$  of the protein for COL and HIN, respectively. This difference is explained by a high content of arginine (4 nitrogen atoms per arginine molecule) in HIN clones. For COL, the proteins contained, on average: 23.3% glutamic acid, 15.7% proline, 14.3% arginine, 7.9% aspartic acid, while for HIN: 23.5% glutamic acid, 2.3% proline, 35.5% arginine, 7.9% aspartic acid. A linear correlation was found between the total nitrogen content and the level of arginine in cassava roots. Root protein content based on amino acid profile varied between 1.0% and 2.8% in the clones analyzed. Screening of varieties by the total nitrogen content leads to identify clones that are richest in arginine and not necessarily the richest in protein. Nitrogen alone, therefore, is not adequate to predict protein content.

## A REAPPRAISAL OF NITROGEN-TO-PROTEIN CONVERSION FACTORS IN CASSAVA ROOTS

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### Introduction

Cassava roots are valued for their energy contribution to the diet. However, they are remarkably devoid of other nutrients like minerals, vitamins and proteins. Large efforts have been made to improve the nutritional quality of cassava roots. Significant progress has been achieved increasing pro-vitamin A carotenoids, and early on large variation in protein content based on the indirect method of quantifying N through Kjeldahl (**Chávez et al., 2005; Ceballos et al., 2006**). However this early work on protein content mentioned the uncertainty related to the N-to-protein conversion factor (**Solsulki & imafidon ; 1990**). This article answers this issue and assesses the real potential to breed for enhanced protein content in cassava roots through conventional breeding.

### Materials and Methods

Two sets of materials were analyzed. The first set was a group of 11 clones whose levels of N in the roots were average based on the available data (**NN**). These clones were grown in Agrovez Farm in Jamundí, Colombia. Root samples of 15 high-N clones (**HN**) were also analyzed. For eight of these clones two different samples had been obtained in 2006 and 2010. These samples were analyzed independently and their results averaged. For the remaining seven HN clones root samples from 2010 were only available. N was initially quantified through Kjeldhal and then by the Dumas method (elementary analyzer THERMOQUEST- CN, **NF ISO 13878, 1998, Simmonne et al. 1997**).

Total amino acids analysis was carried out using a Biochrom 30 amino acid analyser (Biochrom Ltd., Cambridge, UK). The whole process takes about 90min per sample. The quantification was carried out comparing peaks areas with a complete standard including 27 amino acids (acidics and neutrals AA, basics AA, tryptophane, all purchased from Sigma, Saint-Quentin Fallavier, France) .Norleucine (250 nmol mL<sup>-1</sup> in sodium citrate buffer, 0.2 M, pH 2.2) was used in addition as internal standard. (**Loscos et al., 2008**)

## Results

**Table 1** presents a summary of the two types of genotypes analyzed. The HN group had an average N content of 0.85% and the highest value detected in roots was close to 1.30%. The NN clones had an average of 0.47 which is similar to the one reported by **Chávez et al., in 2005** for the entire core collection of CIAT (0.49%). The conversion factors of total nitrogen into protein calculated from amino acid profiles was  $3.6 \pm 0.9$  for NN and  $2.8 \pm 0.2$  for HN in agreement with **Hock-kin & Truong (1996)**. Total nitrogen from protein was similar in NN (53.7%) to that in HN (52.6%). The remaining N ( $\approx 46\%$ ) corresponds to ammonium ions, nucleic acids, or other non-protein nitrogenous molecules. Nitrogen content of the protein was  $15.1 \pm 1.2$  and  $19.1 \pm 0.7\%$  for HN and HIN, respectively. This difference is explained by a high content of arginine (4 N atoms per amino acid molecule) in HN clones. For NN, the proteins contained, on average: 23.3% glutamic acid, 15.7% proline, 14.3% arginine, 7.9% aspartic acid, while for HN:

23.3% glutamic acid, 2.4% proline, 35.5% arginine, and 7.8% aspartic acid. There was a strong correlation between total N content and the level of arginine in cassava roots. Root protein content based on amino acid profile varied between 1.0% and 2.8% in the clones analyzed. Screening of varieties by the total nitrogen content leads to identify clones that are rich in arginine and not necessarily outstanding in total protein content. Nitrogen alone, therefore, is not adequate to predict protein content.

**Table 1.** Nitrogen content and amino acids (expressed as % of total protein content) quantified in a set of cassava clones with normal (11 clones) and high levels of nitrogen(15 clones) in the roots. For the high-N group minimum, maximum and average levels are also provided.

Amino acid and Other parameters	“Normal-N” (Agrovez)	High-N clones		
		Minimum	Maximum	Average
Cysteic Acid (%)	0.2	0.0	0.6	0.3
Aspartic acid (%)	7.9	5.5	9.1	7.8
Threonine (%)	2.0	1.7	2.9	2.2
Serine (%)	2.2	2.0	5.1	3.0
Glutamic acid (%)	23.3	18.9	28.5	23.3
Glycine (%)	2.3	1.4	2.5	1.9
Valine (%)	6.5	3.3	6.0	4.4
Alanine (%)	0.8	0.6	1.3	0.9
Cystine (%)	3.5	2.2	3.3	2.7
Methionine (%)	1.2	1.1	2.3	1.6
Isoleucine (%)	2.5	1.3	1.9	1.5
Leucine (%)	3.9	2.0	3.4	2.5
Tyrosine (%)	2.2	1.5	2.5	2.0
Phenylalanine (%)	1.4	1.2	1.6	1.4
Histidine (%)	3.2	2.3	3.9	3.0
Lysine (%)	6.9	2.8	5.0	3.6
Arginine (%)	14.3	28.7	43.0	35.5
Proline (%)	15.7	2.0	3.0	2.4
N total by Dumas (%)	0.469	0.68	1.29	0.85
N from AA (%)	0.239	0.38	0.53	0.44
Protein = Sum (AA) (%)	1.57	2.00	2.82	2.32
N ratio in protein (%)	15.1	17.91	20.76	19.10
N from protein (%)	53.7	37.63	58.01	52.60
conversion factor N:P	3.6	2.10	3.07	2.75

## References

Chávez, A.L., T. Sánchez, G. Jaramillo, J.M. Bedoya, J. Echeverry, E.A. Bolaños, H. Ceballos and C. A. Iglesias (2005). Variation of quality traits in cassava roots evaluated in landraces and improved clones. *Euphytica* 143:125-133.

Ceballos H., T. Sánchez, A.L. Chávez, C. Iglesias, D. Debouck, G. Mafla and J. Tohme (2006). Variation in crude protein content in cassava (*Manihot esculenta* Crantz) roots. **Journal of Food Composition and Analysis** 19:589-593

Hock-Hin Y. & Truong Van-Den (1996). Protein Contents, Amino Acid Compositions and Nitrogen-to-Protein Conversion Factors for Cassava Roots. **Journal of the Science of Food and Agriculture**, 70(1):51-54.

Loscos N., Ségurel M., Dagan L., Sommerer N., Marlin T., Baumes R. (2008). Identification of S-methylmethionine in Petit Manseng grapes as dimethyl sulphide precursor in wine. **Analytica Chimica Acta**, 621:24–29.

Simonne A H, Simonne E H, Eitenmiller R R, Mills H A, Cresman C P (1997). Could the Dumas method replace the Kjeldahl digestion for nitrogen and crude protein determinations in foods? **Journal of the Science of Food and Agriculture**, 73:39-45.

Sosulski F.W. & Imafidon G.I. (1990) Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. **Journal of Agricultural and Food Chemistry**, 38(6):1351-1356